Single-cell dissection of transcriptional heterogeneity in human colon tumors.

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Public Summary:
Tumors are complex tissues in which distinct populations of cancer cells coexist and intermingle. The “cancer stem cell” theory proposes that, in tumor tissues, cancer cells undergo a progressive differentiation process similar to that observed in normal tissues, where a subset of immature stem/progenitor cells expand and diversify to generate multiple types of specialized mature cells. In essence, the “cancer stem cell” theory views cancer tissues as a “distorted version” or “caricature” of normal developmental processes. Two aspects of the “cancer stem cell” theory have been difficult to investigate: (i) the extent to which the cellular diversity of cancer tissues mirrors that of normal ones, including the coexistence of immature cells with stem/progenitor properties mixed with mature specialized cells; (ii) the molecular mechanism that underlies this diversity. The goal of our investigations was to address these basic questions, in order to develop better prognostic and therapeutic tools for cancer patients. In the first part of this study we used "single-cell PCR gene-expression analysis" (SINCE-PCR) to compare the cellular composition of human colon cancer tissues with that of normal colon epithelia. Starting from surgical specimens obtained from Stanford Hospital, we isolated single-cell suspensions of colon epithelial cells and used flow cytometry to sort them one-by-one, into arrays of single (n=1) cells. We then used SINCE-PCR to analyze each individual cell for the expression of multiple genes in parallel, and used statistical “clustering” algorithms to associate cells with similar gene-expression profiles. The results of this experiment allowed the discovery of new cell populations of the colon epithelium and of new markers to label them. Most importantly, we found that colon carcinomas, indeed, do contain multiple cell populations whose gene-expression profiles closely mirror those of both mature and immature cell types of normal colon epithelia. The second aim was to test whether the cellular diversity observed in cancer tissues was generated by a differentiation process reminiscent of a stem-cell hierarchy, as opposed to the coexistence of cell populations bearing different types of DNA mutations. In this second experiment, we injected single (n=1) human colon cancer stem cells into immunodeficient mice, to generate “monoclonal” tumors (i.e. composed by cells originated from the expansion of a common ancestor). Remarkably, the “monoclonal” tumors recapitulated the full cellular diversity of their parent tissues, indicating that cancer cell diversity can be largely explained as the result of a stem-like multi-lineage differentiation process. Finally, we investigated whether differences in cancer cell composition could translate into different survival outcomes for cancer patients. Using our SINCE-PCR data to identify genes selectively expressed by mature cell types, we developed a two-gene classification system (KRT20 vs. either CA1, MS4A12, CD177 or SLC26A3) that allowed for stratification of colon cancer patients by “gene-expression groups” with different hazard-ratios. Importantly, the prognostic capacity of this novel classification system appeared to outperform traditional prognostic parameters, such as pathological grade, and is currently investigated for clinical applications.

Scientific Abstract:
Cancer is often viewed as a caricature of normal developmental processes, but the extent to which its cellular heterogeneity truly recapitulates multilineage differentiation processes of normal tissues remains unknown. Here we implement single-cell PCR gene-expression analysis to dissect the cellular composition of primary human normal colon and colon cancer epithelia. We show that human colon cancer tissues contain distinct cell populations whose transcriptional identities mirror those of the different cellular lineages of normal colon. By creating monoclonal tumor xenografts from injection of a single (n = 1) cell, we demonstrate that the transcriptional diversity of cancer tissues is largely explained by in vivo multilineage differentiation and not only by clonal genetic heterogeneity. Finally, we show that the different gene-expression programs linked to multilineage differentiation are strongly associated with patient survival. We develop two-gene classifier systems (KRT20 versus CA1, MS4A12, CD177, SLC26A3) that predict clinical outcomes with hazard ratios superior to those of pathological grade and comparable to those of microarray-derived multigene expression signatures.

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